A change in color of aging mycorrhizal roots of *Tilia americana* formed by *Cenococcum graniforme*

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Received February 27, 1970

PARK, J. Y. 1970. A change in color of aging mycorrhizal roots of *Tilia americana* formed by *Cenococcum graniforme*. Can. J. Bot. **48**: 1339–1341.

It is accepted that mycorrhizae formed by *Cenococcum graniforme* (Sow) Ferd. & Winge are characteristically black. From the data reported here it is apparent that, contrary to this view, mycorrhizal roots formed by this symbiotic fungus may also be brown as well as white. Anatomical studies have led to the conclusion that the differences in color of mycorrhizal roots formed by *C. graniforme* are related to the physiological state of the symbiotic relationship. Apparently, the white mycorrhizae reflect the early period of the symbiosis and the black mycorrhizae indicate the period at which the symbiotic association begins to deteriorate. Experimental evidence indicates that mycelium of *C. graniforme* in pure culture changes color through aging and also through certain changes in nutritional conditions.

Introduction

According to Ferdinandsen and Winge (1925), *Cenococcum graniforme* is a black, mycorrhiza-forming fungus which develops a lustrous mantle around the mycorrhizal rootlet. From this mantle radiate jet-black hyphae. The fungus is a symbiotic associate with many tree species of angiosperms and gymnosperms in Europe and North America. The taxonomy, physiology, ecology, and geographical distribution of this fungus have been studied by numerous workers (Melin 1927; Hatch 1937; Lihnell 1942; Mikola 1948; Keller 1952; Trappe 1962*a*, 1962*b*, 1964, 1966; Zak and Bryan 1963; Zak and Marx 1964).

The data reported in this paper are derived from studies on mycorrhizae in relation to basswood seedling establishment under specified ecological conditions.

Materials and Methods

The mycorrhizal roots were obtained from 1- to 5-year-old basswood seedlings growing in natural, mixed hardwood forests consisting of maple, beech, birch, elm, and oak. The seedlings were collected from May to August in 1968 from six different locations in southern Ontario. A total of 300 seedlings were used. The excavated seedlings, together with adhering soil, were shipped by air express to the Sault Ste. Marie laboratory in an ice chest.

Isolation of the symbiotic fungi from mycorrhizae in pure culture was achieved by using the sterilization apparatus designed by Slankis (1958). Before sterilization, selected root tips were excised, washed with sterile water, transferred into the sterilizing compartment of the dish, and rinsed for 3 min with dripping water. The root tips were sterilized with 0.1% $HgCl_2$ for 15 s, at a flow rate of two drops per second, and then were washed with sterile dripping water for 3 min. The sterilized rootlets were introduced into individual test tubes containing Hagem agar medium so that only the basal part of the rootlet was submerged in the medium (Modess 1941). The test tubes were closed with cotton plugs and stored at room temperature (20 to 25 °C). The developing hyphae were examined regularly as they emerged from the rootlets.

For the studies of hyphal characteristics, a portion of aerial hyphae that had emerged from the rootlet was transferred onto three different media for further development: Hagem agar, water agar containing 10% glucose in Petri dishes, and wheat grain medium in a flask.

To prepare the wheat grain medium, the grain was soaked in water, boiled for ½ h, spread on a dry cloth to drain, and autoclaved in conical flasks at 15 p.s.i. for 30 min. The autoclaved flasks were inoculated with the pure culture, incubated at 25 °C, and shaken once a week.

Results and Discussion

Morphological and Anatomical Characteristics of Basswood Mycorrhizae

The morphological and anatomical studies of basswood mycorrhizae revealed three major types of mycorrhizal roots. Type 1 was white. The thin mantle consisted of hyaline hyphae, and an established Hartig net occupied only the outer layer of the cortex (Fig. 1). Type 2 was yellow-brown with a thickened hyphal mantle and a Hartig net that extended nearly to the endodermis (Fig. 2). Type 3 was black, and jet-black hyphae radiated from the outer layer of the pseudoparenchymatous mantle (Fig. 3). A thick Hartig net extended to the endodermis, and intracellular hyphae were present in the cortical cells. When the cortical cells finally began to shrink, the mantle started to collapse; this type of mycorrhizae seemed to reflect the last stage of

the intracellular infections. The intracellular hyphae appeared as granular bodies filling the cells. In later stages, the hyphae appeared as irregular, string-like bodies.

Texture and Color Characteristics of the Symbiotic Fungi in Pure Culture

The aerial hyphae of the fungal symbionts emerged from sterilized mycorrhizal roots within 1 to 6 weeks; and in almost 50% of the cultures, these aerial hyphae were yellow. After the aerial hyphae had been transferred to Hagem agar in Petri dishes, 99 pure cultures were obtained. A typical colony of *C. graniforme* is shown in Fig. 4. Forty-five of these cultures conformed to the taxonomic characteristics described by Ferdinandsen and Winge (1925), Hatch (1937), Lihnell (1942), and Trappe (1962*a*) for *C. graniforme*. However, only 18 of them were derived from the characteristic *Cenococcum* mycorrhizae (type 3), whereas 11 of them originated from type 1 mycorrhizal roots and 16 from type 2 mycorrhizae. The aerial hyphae that emerged from the surface of the 45 sterilized rootlets were all yellow. When these aerial hyphae were transferred onto Petri dishes, for further development, the resulting mycelia turned a brown that ranged from dark lavender to dark brown or black as the mycelia aged. The black, setaceus, aging mycelia on Hagem agar often produced abundant thin, ramified, hyaline or lemon hyphae, which appeared at the periphery or center of the mycelial colony. When lemon or hyaline hyphae were transferred to fresh medium, they became thicker and again turned black.

In Hagem agar, *C. graniforme* always formed a convoluted center in the colony from which radiated thick hyphae, 5 to 10 μ in diameter. Hyaline or yellowish hyphae were also produced when inoculum taken from an old culture with dark, setaceus mycelium was transferred to a medium consisting of water agar and 10% glucose. The newly formed hyphae grew faster horizontally and decreased in diameter to less than 4 μ. The convoluted center of the newly formed colony was lacking.

When dark, setaceus mycelia were transferred to a commercial mushroom spawn medium containing wheat grain, thin, light brown hyphae were produced on the pericarp of the grains. The growth rate of these hyphae was greater than that of the same isolates growing on a synthetic medium. Mikola (1948) has similarly observed a change in hyphal color related to the age of cultures and to variations in the amount of nutrients in the medium.

Conclusions

Mycorrhizal roots associated with *C. graniforme* can be different in color; this finding is contrary to the general belief that these mycorrhizae are always black. *Cenococcum* mycorrhizae change color as they age. The early stage of the symbiosis is characterized by the white and brown of the mycorrhizal roots, whereas the black *Cenococcum* mycorrhizae characterize the latest stage of the aging of the symbiotic relationship. This is indicated by the restricted Hartig net penetration in the cortex characteristic of white and brown *Cenococcum* mycorrhizae; whereas in the "typical" black mycorrhizae, a deterioration of the symbiotic relationship in varying degrees is reflected by the penetration of the Hartig net down to the endodermis, by extensive intracellular infections, and, finally, by the collapse of the hyphal mantle.

Acknowledgment

Thanks are extended to Dr. J. Trappe, who has kindly shared his knowledge in the identification of fungal isolates.

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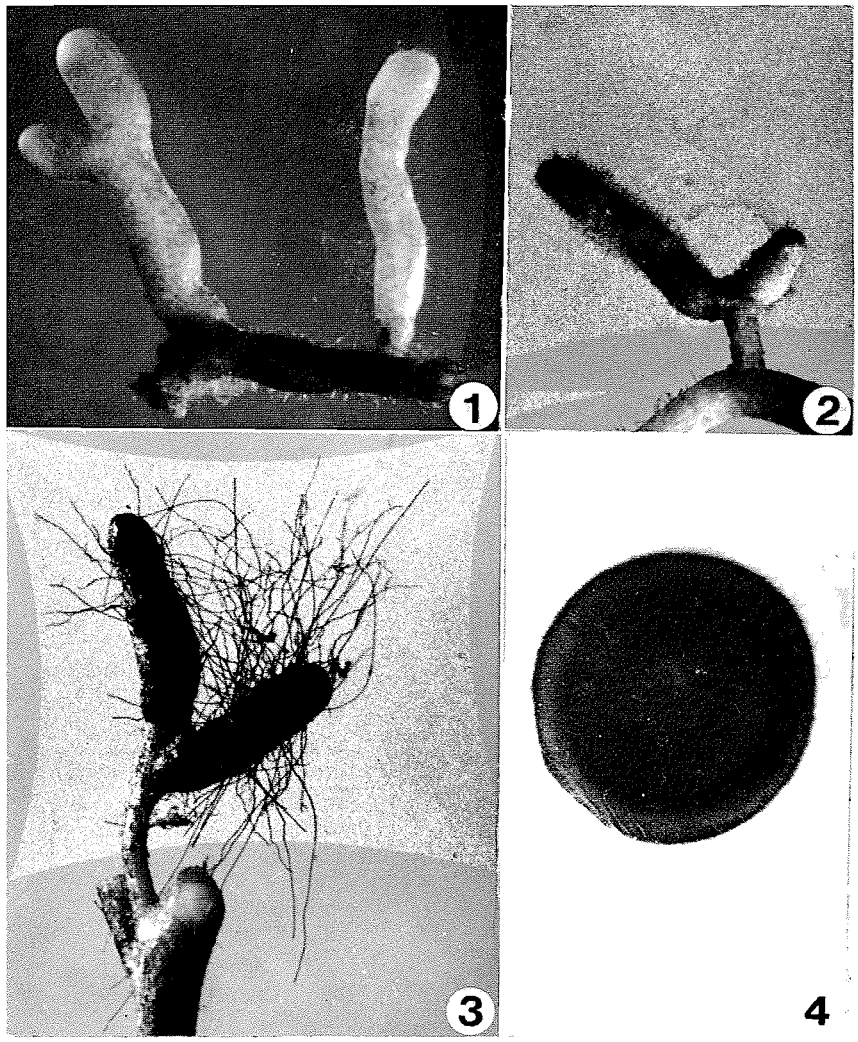
PLATE I



FIGS. 1–4. (× 25) Various types of *Cenococcum* mycorrhizal rootlets and a pure culture colony. Fig. 1, type 1 (white). Fig. 2, type 2 (brown). Fig. 3, type 3 (black). Fig. 4, a typical *C. graniforme* colony on Hagem agar.

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